CERTIFICATION OF EXPRESS MAILING

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR Section 1.10 on September 18th, 2006, Express Mail Label No. ER 262334313 US ,and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

Margaret M. Smart — 55,626 (Reg. No.)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
SMART et al.

Serial No.: 09/816,472 — Group Art Unit: 3736

Filed: March 26, 2001 — Examiner: Robert Nasser

For: SILICON MICROPROBE WITH INTEGRATED BIOSENSOR — Attorney Docket No. Kum11Sil.Prb

DECLARATION UNDER 37 CFR §1.131 OF WILSON SMART

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Wilson Smart, declare and state that:

1. I am one of the inventors of the above identified patent application.

2. I am the President and Chairman of the Board of Kumetrix, Inc., the assignee of the invention that is the subject of the above-identified application.

3. This declaration is to establish conception of the invention in the present application in the United States at a date prior to June 2, 2000, which is the earliest priority date of International Publication No. WO/ 01/9390 A1, which was applied by the Examiner, and diligence in reducing this invention to practice from the date of conception to June 2, 2000. The document attached as Exhibit A is a true photocopy of a signed page from a bound laboratory notebook, describing one embodiment of our invention. The document attached as Exhibit B is a true photocopy of a facsimile received from our attorney outlining the invention in a draft patent application. The number of the notebook has been redacted, as have the dates on the notebook and the facsimile, but these dates are prior to June 2, 2000.

4. I diligently worked on the biosensor microprobe from the date of conception until at least June 2, 2000.

5. Reduction to practice of silicon microprobe occurred at a date prior to June 2, 2000. This reduction to practice is evidenced by the attached document (Exhibit C) which is a true photocopy of an excerpt from a Final Technical Report entitled "Integrated Sampling Device for Microfluidic Bioanalysis Systems submitted to the Defense Advanced Research Projects Agency pursuant to Contract DAAH 01-97-C-R113. The date of this Report, which has been deleted, is prior to June 2, 2000. Several other dates have also been removed from this Report, all dates are prior to June 2, 2000.

6. As set forth the first page of the excerpted Report, several different solid microneedle (i.e. microprobe) geometries varying in width and shape were fabricated fronm single-crystal silicon wafers for experimental evaluation of needle strength. The needles were then subjected to fracture testing, and finite element analysis of the best-performing needles used to further improve needle design. Processing techniques to increase fracture toughness were also developed . The solid microneedles were tested for pain perception on human subjects under the direction of Dr. Nancy Bohannon. Summarized results of these pain perception trial are shown on the last page of the excerpt. Photographs and a finite analysis plot on page 2 of Exhibit C show solid microprobe designs disclosed in claims 1-8, and 10-11.

7. Because Kumetrix is a very small company, additional government funding for biosensor miniaturization and integration was sought and obtained as soon as possible following conception of the invention. Five proposals were submitted to government agencies, the first proposal within three months of the date of the conception. Experimental biosensor work began upon notice of award, and continued without interruption until at least June 2, 2000. An electrochemist with experience in microbiosensors was added to the staff.

8. Evidence of diligence in reduction to practice of the complete biosensor microprobe is set forth in the attached document (Exhibit D) which is true photocopy of an excerpt from a Monthly Progress Report, Report 5 entitled "Continuous Monitoring of Tissue Blood Lactate in the Field" submitted to the US Army pursuant to Contract DAMD17000-C-0008". Page 1 of Exhibit D shows the miniaturized biosensor electrodes deposited on a single-crystal silicon wafer. The portion of the electrode system which fits on the finished probe is shown attached to larger electrodes for ease of handling during biosensor development. The wafer can be plasma etched etched to liberate the individual microprobes. The figure on page Exhibit D of the document is a cyclic voltammogram of the miniaturized electrode structure (1,000 μm^2) demonstrating its electrochemical activity. The date of this Report is May 24, 2000 just prior to June 2, 2000. Work continued on the biosensor microprobe under this Contract and under other funding until at least June 2, 2000.

9. I declare further that all of the statements herein of my own knowledge are true; and all statements made on knowledge and belief are believed true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the present application and any patent issuing thereon.

Date: Sept. 18, 2006

Wilson Smart

Kumetrix, Inc.

29524 Union City Blvd.,
Union City, CA 94587

LABORATORY NOTEBOOK

Notebook No.: _____

Assigned to: Gene Orloff

Date _____

EXHIBIT A

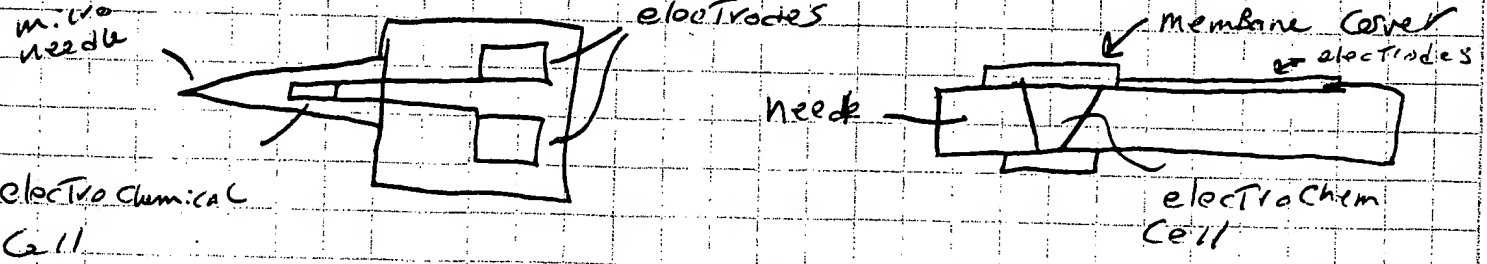
PROJECT micro needle Concepts

Notebook No. _____

Continued From Page _____

I have discussed with Kumar the possibility of designing a new device other than the current micro needle design. The motivation for my new idea of blood analysis stems ~~the~~ from the difficulty in obtaining blood from a microneedle poke. However I have noticed small amounts of blood present on the dummy needle tips after poking has occured. The idea is to utilize this blood. My initial suggestion was a membrane which would absorb the fluid & then perform an optical analysis. Kumar warned against this idea, ~~this~~ due to difficulty in working with small membranes. This discussion led to a further ~~idea~~ concept, performing an electrochemical analysis on this small amount of blood or fluid after poking that is deposited on the needle.

This concept was discussed with Wilson Smart at some length and some initial plans for a micro needle with an electrochemical glucose sensor were drawn out



Work will be starting in the near future to investigate the feasibility of these devices.

Continued on Page

Read and Understood By

Signed Date

Signed Date

FINAL TECHNICAL REPORT

INTEGRATED SAMPLING DEVICE FOR MICROFLUIDIC BIOANALYSIS SYSTEMS

Sponsored by

Defense Advanced Research Projects Agency (DOD)
Microsystems Technology Office

DARPA Order No. E612, Amdt. 17

Issued by U.S. Army Aviation & Missile Command Under

Contract #DAAH01-97-C-R113

Name of Contractor:
Smart Instruments (sole proprietorship)
Now incorporated as Kumetrix, Inc.

Principal Investigator:
Dr. Wilson Smart (Extension 600)

Business Address:
29524 Union City Blvd.
Union City, CA 94587-1245
Phone No: (510) 476-0950, Ext. 100
FAX No: (510) 476-0953

Senior Project Engineer:
Kumar Subramanian (Extension 500)

Effective Date of Contract:

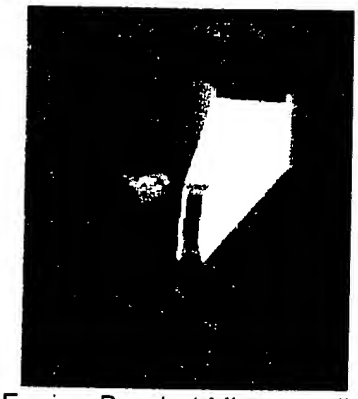
Short Title of Work:
Microfluidic Bioanalysis

Contract Expiration Date:

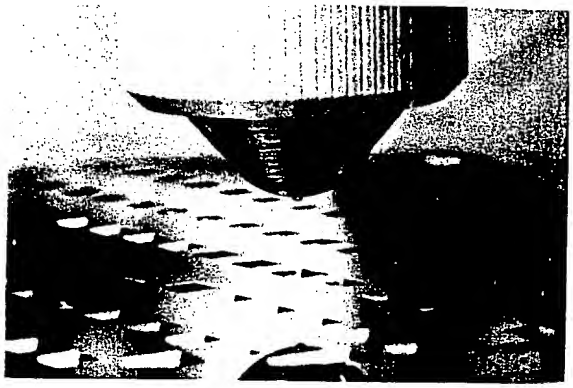
Reporting Period:

EXHIBIT C

shape and the window is formed by plasma etching. A close-up view of a fusion bonded needle is shown below on the left. The next step is to dispense reagents into the microcuvette and dry them. The reagent dispense operation is shown below on the right.

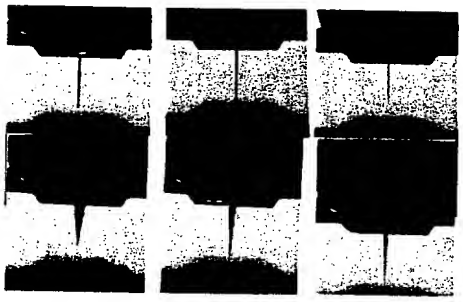


Fusion Bonded Microneedle



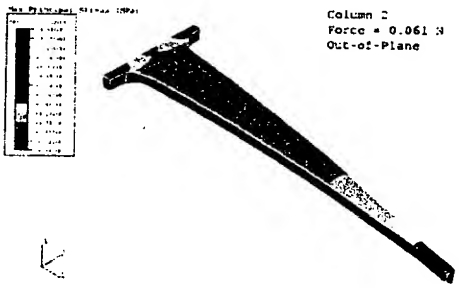
Reagent Dispense

Device Design and Analysis:



Various needle geometries have been designed and fabricated in order to determine the mechanical strength associated with the needle parameters. This photograph illustrates several of these needle geometries. The needles were etched to a thickness of 50 μm and vary in width from 500 μm at the base to 25μm at the tip.

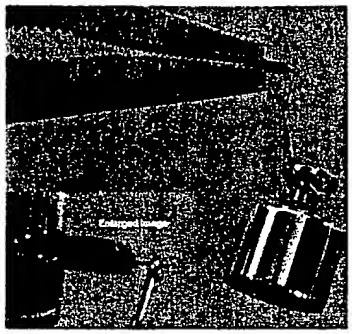
Finite element analysis (FEA) was performed to identify the theoretical fracture and deflection properties of a number of needle geometries, and compare them with experimentally measured values. A stress distribution plot of a needle geometry is shown. Comparison of the actual fracture data and the theoretical stress analysis were in very close agreement. These results have enabled us to use finite element simulations as a tool for further design of needle geometries as a means to maximize strength.



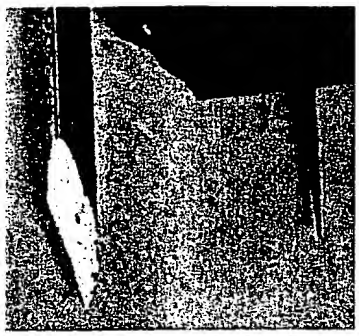
Furthermore, we have recently investigated various processing techniques in order to further strengthen the silicon microneedles. Results obtained from this work have allowed us to greatly increase the critical forces and displacements which the needle can withstand prior to failure. The most promising processing results were obtained by growing a thermal oxide on the silicon cantilever, and from subsequently removing this oxide film in buffered oxide etchant (BOE). Both processing steps resulted in a considerable increase in critical force of the device.

Device Testing: The silicon microneedle is the key innovative feature of this technology. Painless blood sampling requires that these needles be tough and flexible to avoid brittle fracture, and that they have small cross-sections to be painless. Physical testing of the silicon needle strength is fundamental to the process of developing a painless blood-sampling device. An understanding of the forces and displacements that the needles can withstand, as well as the location of needle fracture upon overloading, allowed the research team to identify the most promising designs and to make the necessary changes to optimize needle strength, yet remain

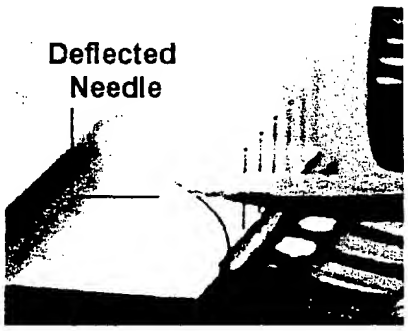
on a scale that will prove painless to the user. The ability of the needle to puncture human skin and to withstand the reaction forces encountered in doing so was also measured. The high degree of success we have achieved in meeting our goals is illustrated in the figures below



Suspended Ten Gram Weight

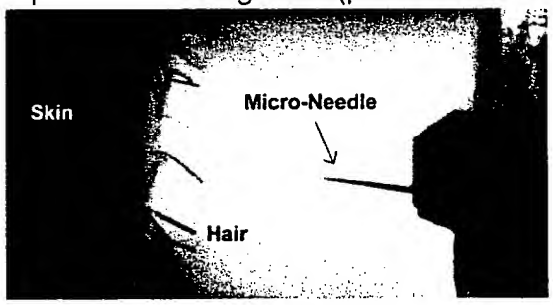


Conventional Lancet vs. Kumetrix MicroNeedle



Needle Flexibility

Skin Puncture Testing: Initial skin puncture testing was performed with the cooperation of a plastic surgeon during a face lift. Skin samples several square centimeters in area were brought directly from the operating table immediately after removal from the patient, in order to maintain the quality of the skin and minimize blood clotting. The skin was repeatedly punctured at different locations with three needle geometries and the forces required to penetrate the skin were recorded. The data from these tests were compared to buckling force (parallel to the needle axis) test data in order to determine the capability of puncturing human skin prior to tests on live human subjects. The results show that the force required to puncture the skin is minimal compared to the buckling force required to break the needle. The photograph shows a magnified image of the silicon microneedle in the mechanical strain gauge just prior to being inserted into the skin.



The average force required to puncture the skin as compared to that to fracture the needles is shown below, demonstrating that the needles are indeed strong enough for their intended use.

Puncture Force (Newton)	Buckling Force (Newton)
0.038	0.134

Biocompatibility Testing: Biocompatibility studies of the silicon microneedles were established prior to human subjects testing. Test animal studies to evaluate cytotoxicity, sensitization, irritation, systemic toxicity and hemocompatibility. The tests were conducted at the Northview Pacific laboratory whose animal science program is accredited by AAALAC. The biocompatibilty studies were successfully completed showing no effects on the test animals.

Human Subject Testing: Kumetrix has Institutional Review Board (IRB) approval, and certification from the Office for Protection from Research Risks (OPRR) of the U.S. Department of Health and Human Services, to conduct human subjects testing. Human subject tests were conducted under the direction of Dr. Nancy Bohannon with 41 diabetics and 21 non-diabetics, at St.Luke's Hospital in San Francisco. The results of this clinical trial are very encouraging:

- All modes of use of the microneedles were significantly less painful than the lancet
- In many cases, the microneedle could not even be felt as it was inserted
- Needle breakage was < 0.5% and the broken piece was easily removed

DATA

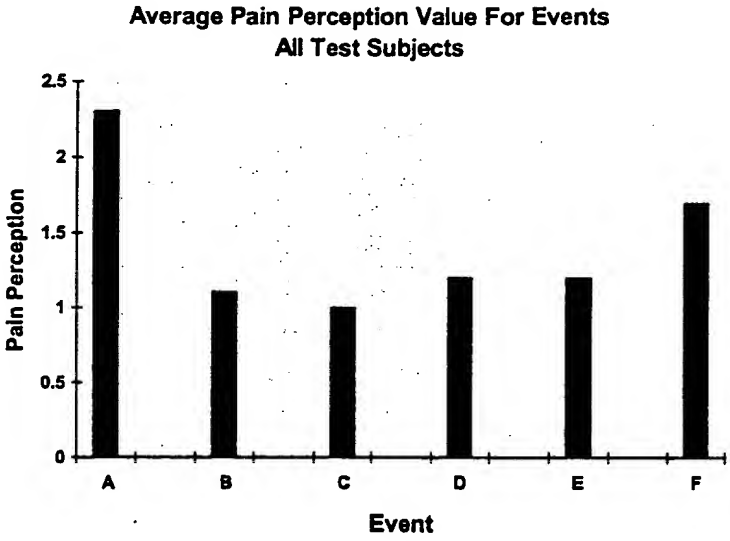
The follow pages show the results of the clinical tests. The table and graphs below show the average pain perception values recorded for each event. The first graph shows data for all test subjects, the second graph displays the results for tests performed on diabetics only.

AVERAGE PAIN PERCEPTION VALUES

Events	A	B	C	D	E	F
All Subjects	2.3	1.1	1.0	1.2	1.2	1.7
Diabetics	2.3	1.1	1.1	1.2	1.2	1.9

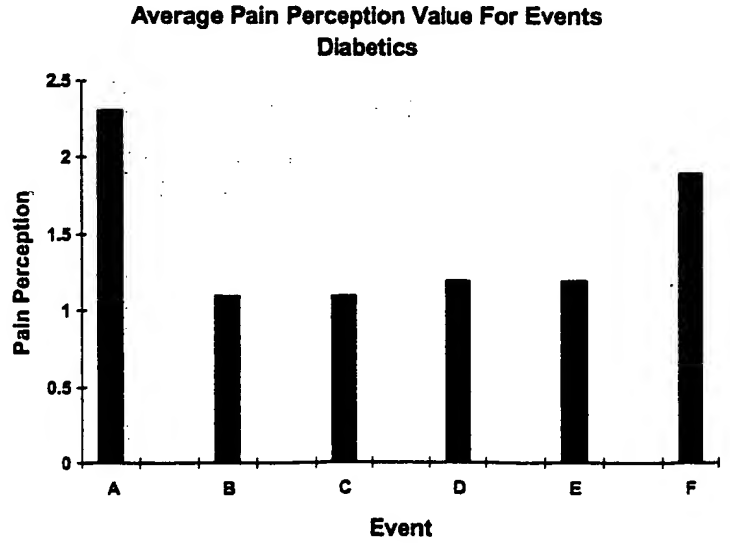
Event Description

A	Finger Stick
B	50µm Poke in Forearm
C	50µm Poke in Forearm
D	100µm Poke in Forearm
E	100µm Poke in Forearm
F	Lancet poke in Forearm



Pain Perception Scale

1	Barely Noticeable
2	Slightly Painful
3	Somewhat Painful
4	Painful
5	Very Painful



MONTHLY PROGRESS REPORT
Report 5

CONTINUOUS MONITORING OF TISSUE BLOOD LACTATE IN THE FIELD

Contract # DAMD17-00-C-0008

US Army Medical Research Acquisition Activity Director
ATTN: MCMR-AA-B

820 Chandler Street
Fort Detrick, MD 21702-501

Name of Contractor:
Kumetrix, Inc.

Business Address:

29524 Union City Blvd.
Union City, CA 94587-1245
Phone No: (510) 476-0950, Ext. 100
FAX No: (510) 476-0953

Contract Expiration Date:

Principal Investigator
Dr. Wilson Smart (Extension 600)

Contracting Representative
Ms. Patricia M Mcallister

Reporting Period:

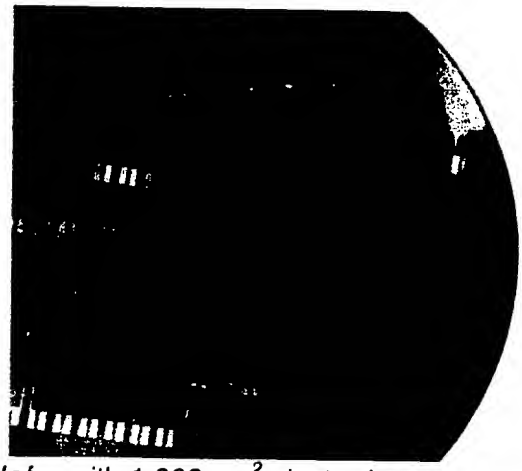
EXHIBIT D

Section 1: Introduction

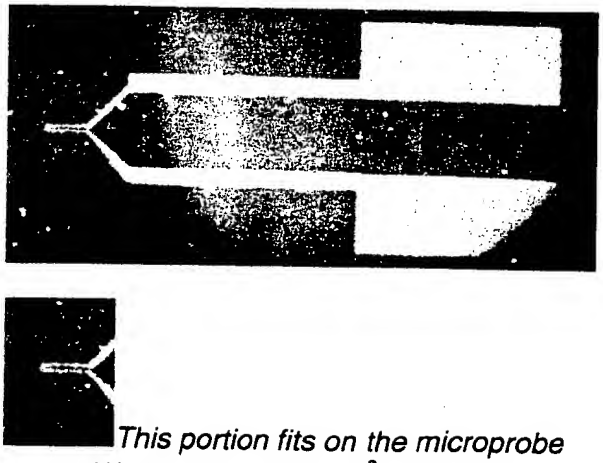
Blood lactate concentration is a sensitive measure of anaerobic metabolism and hence of tissue oxygen deprivation, and is a reliable indicator for assessing trauma, blood loss, and shock in wounded personnel. Development of a rugged, unobtrusive device for continuous monitoring of tissue blood lactate in the field is proposed. Its key component is a silicon microprobe, comparable in cross-section to a human hair, protruding subcutaneously from a small button-like device firmly affixed to the skin with adhesive. An electrochemical lactate sensor integrated into the microprobe continuously measures lactate concentration, and the data are transmitted via a low power telemetry chip to a suitable receiver. This device combines two successful technologies: electrochemical biosensors and silicon microprobes. Biosensors have attained a high level of performance based on more than thirty years of development in many laboratories. Silicon microprobes which possess high strength, flexibility, and fracture toughness are an advanced development from extensive MEMS (microelectromechanical systems) R&D in the proposer's laboratory. Impressive biosensor R&D progress has not yet resulted in commercial devices for *in vivo* applications because other workers in needle biosensors have used conventional configurations and fabrication methods. The strength of this new approach lies in the combination of biosensors and our unique silicon microprobes. By rendering their use easy and painless and sharply reducing the cost, disposable continuous lactate sensors become practical. A design for such devices with a useful operating lifetime of one day, and experimental tasks for their development, are presented. The Phase I Basic Program will demonstrate feasibility by integrating lactate biosensors into silicon, fabricating lactate biosensor microprobes, and performing *in vitro* testing

Section II: Progress

During this work period, miniaturized gold electrodes of 1,000 μm^2 have been fabricated on a 4 inch diameter silicon wafer. The figure below shows one of our patterned wafers along with a close up view of one of the miniature electrodes. At this magnification, the 1,000 μm^2 gold electrodes are not visible, but at higher magnification they can clearly be seen. These electrodes can fit onto our microprobes that are the size of a human hair. Individual electrodes were liberated from the wafer by scoring and cleaving and then cleaned to remove any residual photoresist. Using a microscope and a very fine brush all but the miniature electrodes and the large electrical contact pads were coated with insulating lacquer.



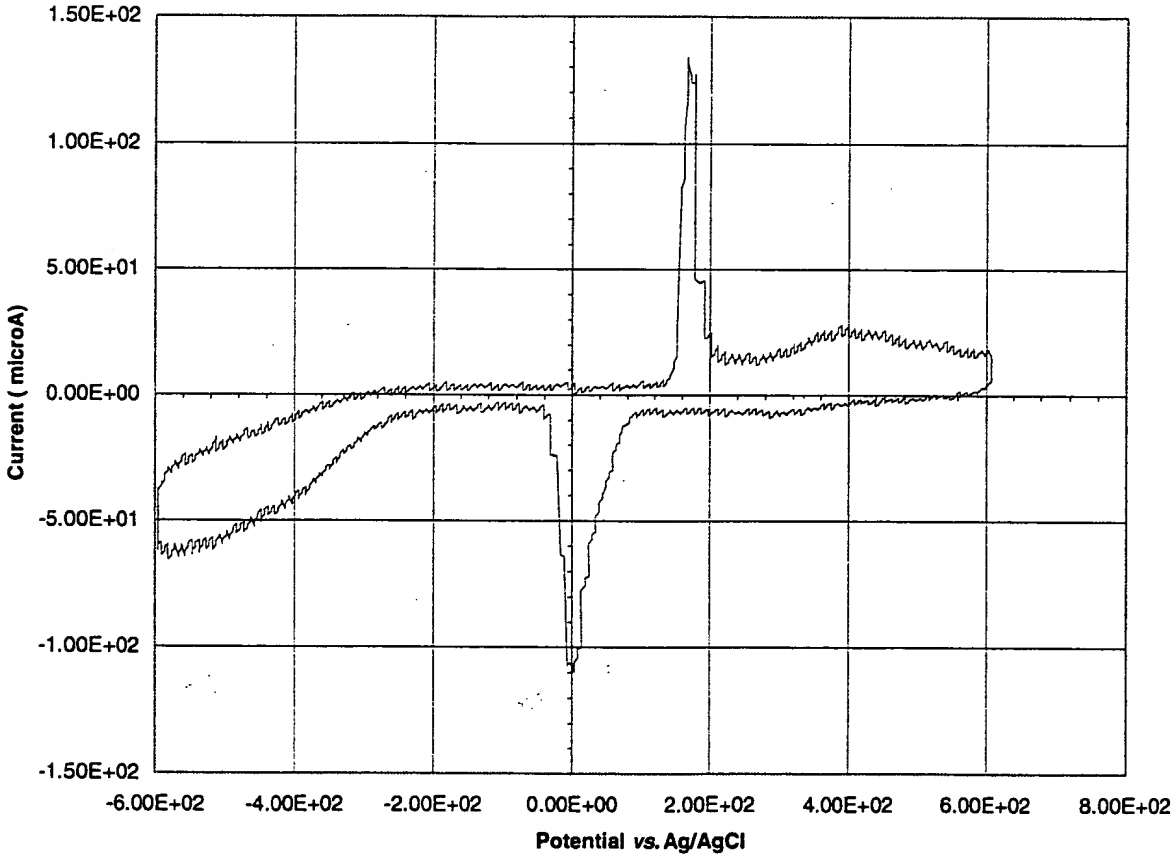
Wafer with 1,000 μm^2 electrodes



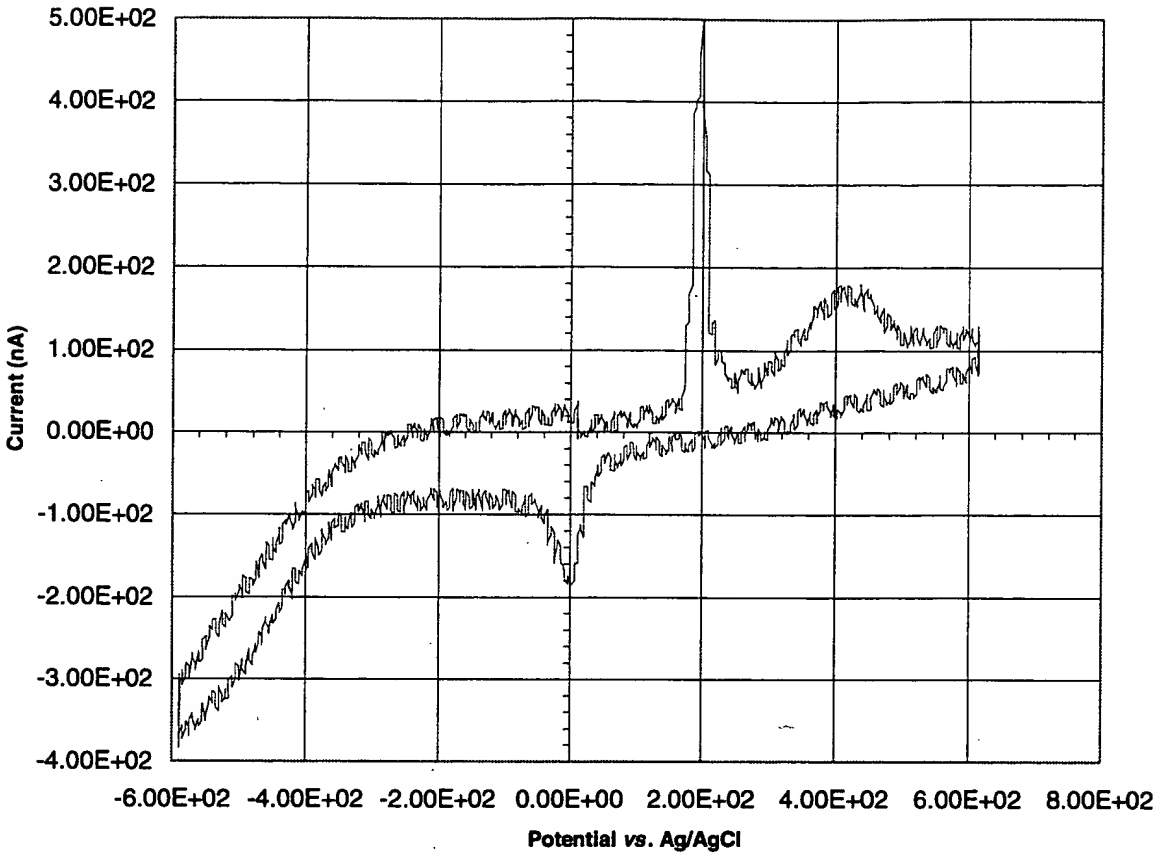
This portion fits on the microprobe

Wafer with 1,000 μm^2 electrodes

The electrochemical behavior of these miniature electrodes was compared with the larger 1 mm^2 (1,000,000 μm^2) electrodes. These miniature electrodes are one thousand times smaller then the larger 1 mm^2 electrodes. This comparison was performed by observing the current output from cyclic voltammetry experiments. The mediator chosen for this comparison is ferrocene monocarboxylic acid, a common electron mediator used in biosensor research, due to its low oxidation potential. Cleaned and prepared electrode structures were placed into our holders and immersed into a 0.5mM solution of ferrocene monocarboxylic acid where the cyclic voltammetry experiment was performed. The next figures show the comparison current between the 1 mm^2 and 1,000 μm^2 gold electrodes. An electrical current of one nanoampere (nanoA) is one-thousandth of a microampere (microA) and is easily measurable with our state-of-the-art electrochemical instrumentation. The significance of this comparison is that even with electrode structures that can fit onto a device the size of a human hair, we observe easily measurable currents, arising from the mediator suitable for electron transfer in enzymatic lactate measurements.



1 mm^2 (1,000,000 μm^2) electrode



1,000 μm^2 electrode

RAJENDER LAW OFFICES
Shyamala T. Rajender, Ph.D., J.D.
Attorney at Law
700 Montgomery Street, 3rd Floor
San Francisco, CA 94111
Tel: (415) 433-0600; Fax: (415) 788-6660
E-Mail: Shyamala@aol.com

DRAFT

TO: Kumar Subramanina, Kumetrix, Inc. 510-476-0953

FROM: Shyamala

SUBJECT: Draft Patent Appln for Biosensor/Microprobe

MESSAGE:

Hi, Kumar: Here, finally, is the draft application for the microprobe (biosensor). There is so much prior art and that even a combination patent giving you enough protection was quite a task. It took a long time to figure out a way around the prior art and still make it broad enough - one of the reasons for the time it took. Please review it carefully, make any changes, additions, deletions, suggestions etc. and send it back to me at your earliest convenience. We will finalize it and have it filed. I will be working at home for the next few days and so callme at home, if you need to get in touch with me (925-736-7410) and fax ((25-736-7375). Just recuperating from gall bladder surgery last Thursday. I should be back in the office sometime next week. If you have any questions, please do not hesitate to call.

Thanks again for your patience and your business.

Warm regards,

Shyamala

DRAFT

EXHIBIT B

DRAFT

COMBINATION SILICON MICROPROBE WITH BIOSENSOR FOR OBTAINING AND ANALYZING BLOOD SAMPLES

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates generally to a silicon microprobe. More specifically, it is concerned with a combination silicon microprobe with a biosensor incorporated thereon for obtaining and analyzing blood samples.

2. Description of the Prior Art

Diabetes mellitus is an insidious disease which affects more than 15 million Americans. About 1.5 million of these are insulin-dependent or Type I diabetic and 12 to 14 million type II or noninsulin-dependent. Both types of diabetes are also considered one of the most prevalent chronic conditions. Chronic, persistently high levels of glucose in blood and in urine are characteristic of diabetics. Although glucose in the urine has been used to monitor glucose levels, the measurement of blood glucose is more reliable and logistically feasible. It has, therefore, become the most commonly followed marker for monitoring the progress of the disease and to determine treatment and control protocols.

While glucose levels are monitored in doctors' offices, clinical laboratories, and hospitals, the most convenient and important is the in-home or self-monitored measurement of glucose levels by the patients themselves to adjust the administration of insulin or hypoglycemics accordingly. This process is known as self-monitored blood glucose (SMBG). Normal glucose levels in the human blood have been established by various health organizations and the World Health Organization, to be in the 70-100 mg/dl range and in the 160-200 mg/dl range after a heavy meal.

There are many products for diabetes related testing of glucose for diagnostic and monitoring purposes. These products range from skin swabs, reagent test strips, portable electronic meters, sensors and other instruments, lancets and needles of various shapes and sizes, syringes and other paraphernalia. Most of the currently available technologies, especially for self-monitored blood glucose measurements, are not very satisfactory

because they all require some kind of deep lancing or finger-stick with the associated pain or sometimes even excessive bleeding.

The smallest prior art lancet or needle currently marketed has a diameter between approximately three-hundred (300 μm) micrometers and approximately five-hundred (500 μm) micrometers and is constructed of a stainless steel material with beveled edges. The needles are packaged in plastic for preservation of sterility and used to pierce the skin for a blood sample. Although there are a number of uses for the blood samples, all lancets have the same function of piercing the skin and producing a blood sample and hence the needle portion of the lancets are very similar from company to company with the only difference being the packaging and the lancet device assembly.

Due to the large diameter of the lancet in the prior art, fingertip lancing is the most painful of diabetes diagnoses. Not only does using a lancet of such large diameter cause extreme pain for the patient, frequent use of lancing the fingertip with a large diameter lancet causes calluses, impairment of the use of hands, psychological trauma and other unpleasant consequences. Calluses on the fingertips means that deeper penetration of the skin on the fingertip will be required over time thereby potentially increasing the pain associated with this method.

Furthermore, once the lancet has recovered the blood sample from the patient, the patient is required to transfer the blood sample to a biosensor for assaying the chemical analytes of the blood sample. Using a biosensor in such a fashion requires that the blood or other biological sample be drawn from the patient by pipetting and not microneedles. Drawing the sample by pipetting can be quite painful for the patient.

It is an object of the present invention, therefore, to provide a combination silicon microprobe with biosensor which obtains and analyzes a blood sample has a diameter between approximately fifty (50 μm) micrometers and approximately two-hundred (200 μm) micrometers.

Another object of the present invention is to provide a combination silicon microprobe with biosensor which is constructed from a silicon material.

Yet another object of the present invention is to provide a combination silicon microprobe with biosensor in which the microprobe penetrates the skin to a depth of less

than two (2 mm) millimeters and produces a small, i.e., less than one (1 μl) microliter, blood sample which can be used for diagnostic testing by the biosensor.

Additional object, advantages and novel features of the invention will be set forth in part in the description and drawings which follow, and in part will become apparent to those skilled in the art upon examination of the following or may be learned by practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

SUMMARY

To achieve the foregoing and other objects and in accordance with the purpose of the present invention as embodied and broadly described herein, the present inventions directed to a microsampling device and method for its construction.

A microprobe device capable of piercing a patient's skin for obtaining a blood sample is provided. The microprobe device comprises a needle device for piercing the patient's skin with the needle device having a first end and a second end. The first end pierces the patient's skin. Sensor means contact the blood sample and analyzes the blood sample for chemical analytes.

In an embodiment of the present invention, the microprobe is constructed from a silicon material. Preferably, the microprobe has a length between approximately two (2 mm) millimeters and approximately two-and-one-half (2 1/2 mm) millimeters. Furthermore, preferably, the microprobe has a thickness of between approximately fifty (50 μm) micrometers and approximately one hundred (100 μm) micrometers.

In another embodiment of the present invention, the microprobe device further comprises a silicon device body connected to the second end of the needle device. Preferably, the silicon device body has dimensions of approximately one (1 mm) millimeter square.

In still another embodiment for the present invention, the sensor means is an electrochemical analyte sensor. Preferably, the electrochemical analyte sensor is an

electrochemical cell with redox mediation. In an alternative embodiment, the electrochemical analyte sensor is an electrochemical cell without redox mediation.

In yet another embodiment of the present invention, the sensor means is located nearingly adjacent the first end of the needle device.

In still yet another embodiment of the present invention, the sensor means is located nearingly adjacent the second end of the needle device. Preferably, the microprobe device further comprises a blood flow channel within the needle device for allowing blood flow from the first end of the needle device to the second end of the needle device.

In another embodiment of the present invention, the microprobe device is a single use device.

In still another embodiment of the present invention, the microprobe device is left within the patient's skin for continuous monitoring by the sensor means.

The present invention further includes a method for obtaining and analyzing blood samples. The method comprises providing a microprobe device having a first end and a second end, connecting sensor means to the microprobe device, drawing a blood sample with the microprobe device, and analyzing the blood sample with the sensor means.

In an embodiment of the present invention, the method further comprises constructing the microprobe device from a silicon material.

In another embodiment of the present invention, the sensor means is an electrochemical analyte sensor. Preferably, the electrochemical analyte sensor is an electrochemical cell with redox mediation. In an alternative embodiment, the electrochemical analyte sensor is an electrochemical cell without redox mediation.

In still another embodiment of the present invention, the method further comprises locating the sensor means nearingly adjacent the first end of the microprobe device.

In yet another embodiment of the present invention, the method further comprises locating the sensor means nearingly adjacent the second end of the microprobe device. Preferably, the method further comprises providing a blood flow channel within the microprobe device for allowing blood flow from the first end of the needle device to the second end of the needle device.

In still yet another embodiment of the present invention, the method further comprises the microprobe device being a single use device.

In another embodiment of the present invention, the method further comprises the microprobe device being left within the patient's skin for continuous monitoring by the sensor means.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a top plan view illustrating a silicon microprobe with *in vivo* biosensor, constructed in accordance with the present invention;

FIG. 2 is a perspective view illustrating the microprobe, constructed in accordance with the present invention;

FIG. 3 is a perspective view illustrating a silicon microprobe with *ex vivo* biosensor, constructed in accordance with the present invention;

FIG. 4 is a side view illustrating the biosensor on the silicon microprobe chips, constructed in accordance with the present invention;

FIG. 5 is a sectional view illustrating an electrochemical cell with redox mediation, constructed in accordance with the present invention;

FIG. 6 is a side view illustrating the electrochemical cell with redox mediation of FIG. 5, constructed in accordance with the present invention;

FIG. 7 is a sectional view illustrating an electrochemical cell without redox mediation, constructed in accordance with the present invention; and

FIG. 8 is a side view illustrating the electrochemical cell without redox mediation of FIG. 7, constructed in accordance with the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As illustrated in FIGS. 1 and 2, the present invention comprises a combination silicon microprobe with biosensor, hereinafter referred to as biosensor microprobe device, indicated generally at 10, for obtaining a blood sample for the measurement of biological materials from the blood sample and analyzing the biological materials from the blood sample. The biosensor microprobe device 10 is preferably fabricated from a boron-doped

silicon wafer and measures biological materials from biological fluids such as glucose in blood or urine, fructosamaine in the blood and the like. The biosensor microprobe device 10 is preferably fabricated from a silicon wafer and is generally described in U.S. Patent No. 5,801,057, by the same inventors of the present application and is hereby incorporated herein by reference.

Basically, the present invention includes the biosensor microprobe device 10 through which blood or other body fluids can be drawn into a small sampling chamber or microcuvette (not shown). Preferably, the microcuvette has a volume of less than one (1) microliter.

Basically, the biosensor microprobe device 10 includes a microprobe 12 and a biosensor 14. The microprobe 12 is a very fine, short needle for piercing the skin of the patient to obtain a small blood sample. Preferably, the microprobe 12 is a silicon lancet having a diameter between approximately fifty (50 μm) micrometers and two-hundred (200 μm) at the base and tapering into a needle point. Furthermore, the microprobe 12 preferably has a length between approximately two (2 mm) millimeters and two-and-one-half (2 1/2 mm) millimeters and a thickness of the microprobe 12 is preferably between approximately fifty (50 μm) micrometers and approximately one hundred (100 μm) micrometers. Preferably, the microprobe 12 will be tapered and the junction with a silicon body device 16 is rounded.

The design of the microprobe 12 of the present invention provides a silicon lancet device that punctures the skin and produces a small, i.e., less than one (1 μl) microliter, blood sample useful for diagnostic testing of the patient's blood. The microprobe 12 of the present invention is substantially painless and inhibits the formation of calluses on the patient's fingertips.

As noted above, the biosensor 14 is connected to the microprobe 12. The biosensor 14 can be mounted on the microprobe 12 (*in vivo*), as illustrated in FIGS. 1 and 2, or mounted on the silicon body device 16 (*ex vivo*), as illustrated in FIG. 3. The silicon body device 16 preferably has dimensions of approximately one (1 mm) millimeter square. With the *in vivo* biosensor microprobe device 10, an the biosensor 14 is connected to the silicon body device 16 by electrically conducting traces 18. The

inventors of the present application noticed that the microneedles and microprobes 12 generally emerge from the skin of the patient covered with a film of blood, suggesting that analyte measurements could be made with an *ex vivo* biosensor 14, if the biosensor 14 is located just outside of the surface of the skin when the probe is fully inserted. With the *ex vivo* biosensor 14, an open channel 20 is etched into the silicon microprobe 12 to direct blood to the biosensor 14. The restrictions on leakage of reagents for an *ex vivo* biosensor 14 are much less strict than for an *in vivo* biosensor 14.

The biosensor 14 for *in vivo* application are fabricated on the surface of the microprobe 12 having dimensions of the order of a few tens of microns. For *ex vivo* use, the biosensor 14 of the present invention can have the same dimensions as the *in vivo* biosensor 14, or the *ex vivo* biosensor 14 can be considerably larger, up to approximately one (1 mm) millimeter square. In single use disposable applications, the microprobe 12 is positioned within the skin and the biosensor 14 is active for only approximately one (1) minute. The short operating lifetime permits great flexibility in design and material. The biosensor 14 for *in vivo* use should preferably not leak reagents and must be biocompatible with human tissue, whereas in the *ex vivo* biosensor 14, some leakage is permissible and biocompatibility with human tissue is not necessarily required.

As illustrated in FIG. 4, the biosensor 14 is mounted on the microprobe 12. For continuous monitoring, a microchip 22 on the biosensor 14 will be packaged with a telemetry chip 24 to transmit analyte data to the insulin pump (not shown) for closed loop control. The biosensor 14 is attached to the skin by an adhesive layer 26 in a location such that normal and daily activities are not impeded.

FIGS. 5 and 6 illustrate the biosensor 14 of the biosensor microprobe device 10 of the present invention being an electrochemical cell 30 with redox mediation. The electrochemical cell 30 includes an anode 32 constructed from a noble metal, silicon, or carbon covered with enzyme and mediator and with thin film membrane for diffusional control. A cathode 34 serves as a reference electrode, such as silver-silver chloride. A protective coating 35 is placed around the anode 32, the cathode 34, and the electrically conducting traces 18. The biosensor 14 further includes an outer membrane 36

positioned on an oxide film 38. An anode membrane 40 surrounds an enzyme and mediator 42 with an electrolyte 44, such as hydrogel.

FIGS. 7 and 8 illustrate the biosensor 14 of the biosensor microprobe device 10 of the present invention being an electrochemical cell 50 without redox mediation. The electrochemical cell 50 includes a first anode 52 with active glucose oxidase enzyme constructed from a noble metal, silicon, or carbon covered with active enzyme and a second anode 53 without active glucose oxidase enzyme constructed from noble metal, silicon, or carbon covered without active enzyme. A cathode 54 serves as a reference electrode, such as silver-silver chloride. A protective coating 55 is placed around the first and second anodes 52, 53, the cathode 54, and the electrically conducting traces 18. The biosensor 14 further includes an outer membrane 56 positioned on an oxide film 58. An anode membrane 60 surrounds an active enzyme 62 with an electrolyte 64, such as Hydrogel.

The biosensor microprobe device 10 of the present invention is advantageous for assaying many chemical analytes for which biosensors have been developed, but can not analyze all blood analytes of clinical interest. These other analytes can be assayed using the microneedle devices, in which a blood sample is drawn and transferred into a silicon microfluidic chip where more complex procedures can be carried out. Examples of such analytical procedures include immunochemistry and DNA analysis, developed in other laboratories on silicon microchips.

An optoelectronic instrument (not shown) can read the optical absorbance of the assay chemistry in the combined lancet device and electrochemical biosensor, calculates glucose concentration, and displays the result. In a preferred embodiment, the optoelectronic instrument is not required because the electrical output of the analyte sensor travels to a telemetry chip in the package for wireless transmission.

The combination of existing electrochemical biosensor technology with silicon microbes to produce biosensor microprobes as with the biosensor microprobe device 10 of the present invention can overcome the deficiencies of "needle electrodes" and allow practical *in vivo* use of biosensors. A single-use disposable silicon biosensor microprobe device 10 can be inserted into the skin, take a reading, and be removed painlessly within

one minute. The biosensor microprobe device 10 of the present invention can replace the finger stick and provide truly painless glucose monitoring.

The biosensor microprobe device 10 of the present invention can have an extended operating lifetime for continuous monitoring without a requirement that the lifetime be several months. For example, extending reliable operating lifetime from one minute to one day produces a useful continuous monitor, because replacement is painless and easy and the cost of the device would be very low, comparable in fact to the test strips in current use. The trauma to the tissue of inserting a microneedle and microprobe according to the present invention is much less than that caused by other types of biosensors.

Many of the deleterious processes (enzyme degradation, revascularization, electrode poisoning , membrane fouling) do not become important in this short period. By placing a new biosensor microprobe device 10 on a different location on the skin every week, reliable and clinically meaningful glucose values would be continuously obtained to provide the signal to an insulin pump for closed loop control of blood glucose.

The foregoing description of the preferred embodiments of the subject invention have been presented for purposes of illustration and description and for a better understanding of the invention. It is not intended to be exhaustive or to limit the invention to the precise form disclosed; and obviously many modifications and variations are possible in light of the above teaching. The particular embodiments were chosen and described in some detail to best explain the principles of the invention and its practical application to thereby enable others skilled in the relevant art to best utilize the invention in various embodiments and with various modification as are suited to the particular use contemplated. It is intended that the invention be defined by the claims appended hereto.

CLAIMS

We claim:

1. A microprobe device capable of piercing a patient's skin for obtaining a blood sample, the microprobe device comprising:

a needle device for piercing the patient's skin, the needle device having a first end and a second end, the first end piercing the patient's skin; and

sensor means for contacting the blood sample and analyzing the blood sample for chemical analytes.

2. The microprobe device of claim 1 wherein the microprobe is constructed from a silicon material.

3. The microprobe device of claim 1 wherein the microprobe has a length between approximately two (2 mm) millimeters and approximately two-and-one-half (2 1/2 mm) millimeters.

4. The microprobe device of claim 1 wherein the microprobe has a thickness of between approximately fifty (50 μm) micrometers and approximately one hundred (100 μm) micrometers.

5. The microprobe device of claim 1 and further comprising a silicon device body connected to the second end of the needle device.

6. The microprobe device of claim 5 and wherein the silicon device body has dimensions of approximately one (1 mm) millimeter square.

7. The microprobe device of claim 1 wherein the sensor means is an electrochemical analyte sensor.

8. The microprobe device of claim 7 wherein the electrochemical analyte sensor is an electrochemical cell with redox mediation.

9. The microprobe device of claim 7 wherein the electrochemical analyte sensor is an electrochemical cell without redox mediation.

10. The microprobe device of claim 1 wherein the sensor means is located nearingly adjacent the first end of the needle device.

11. The microprobe device of claim 1 wherein the sensor means is located nearingly adjacent the second end of the needle device.

12. The microprobe device of claim 11 and further comprising a blood flow channel within the needle device for allowing blood flow from the first end of the needle device to the second end of the needle device.

13. The microprobe device of claim 1 wherein the microprobe device is a single use device.

14. The microprobe device of claim 1 wherein the microprobe device is left within the patient's skin for continuous monitoring by the sensor means.

15. A method for obtaining and analyzing blood samples, the method comprising:
providing a microprobe device having a first end and a second end;
connecting sensor means to the microprobe device;
drawing a blood sample with the microprobe device; and
analyzing the blood sample with the sensor means.

16. The method of claim 15 and further comprising constructing the microprobe device from a silicon material.

17. The method of claim 15 wherein the sensor means is an electrochemical analyte sensor.

18. The method of claim 17 wherein the electrochemical analyte sensor is an electrochemical cell with redox mediation.

19. The method of claim 17 wherein the electrochemical analyte sensor is an electrochemical cell without redox mediation.

20. The method of claim 15 and further comprising locating the sensor means nearingly adjacent the first end of the microprobe device.

21. The method of claim 15 and further comprising locating the sensor means nearingly adjacent the second end of the microprobe device.

22. The method of claim 21 and further comprising providing a blood flow channel within the microprobe device for allowing blood flow from the first end of the needle device to the second end of the needle device.

23. The method of claim 15 and further comprising the microprobe device being a single use device.

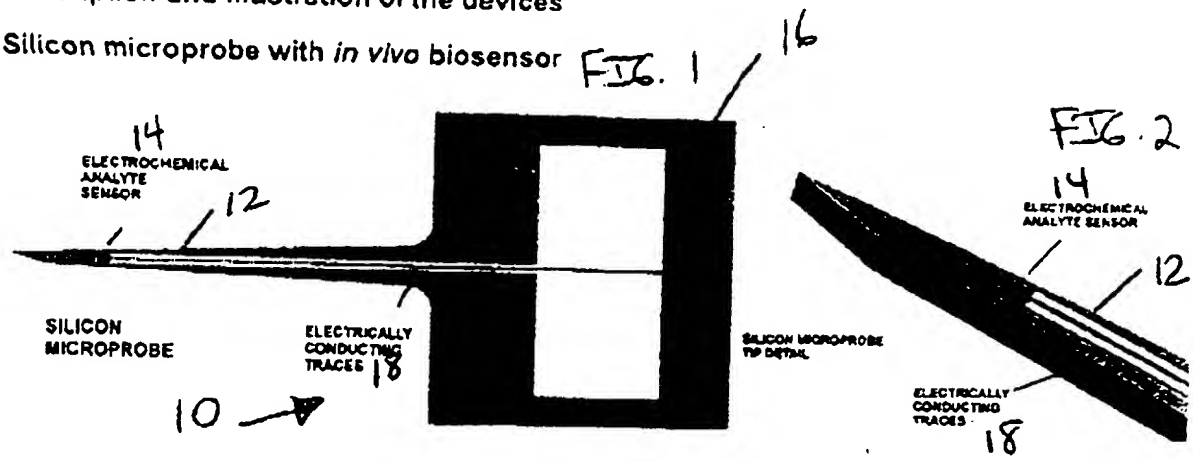
24. The method of claim 15 and further comprising the microprobe device being left within the patient's skin for continuous monitoring by the sensor means.

ABSTRACT

A minimally intrusive and less painful, self-use lancet combination microprobe with biosensor device for the measurement of glucose and analytes in blood are provided. The device of the present invention is a needle device for piercing the patient's skin with the needle device having a first end and a second end. The first end pierces the patient's skin. A sensor mechanism contacts the blood sample and analyzes the blood sample for chemical analytes.

Description and illustration of the devices

Silicon microprobe with *in vivo* biosensor



Silicon microprobe with *ex vivo* biosensor

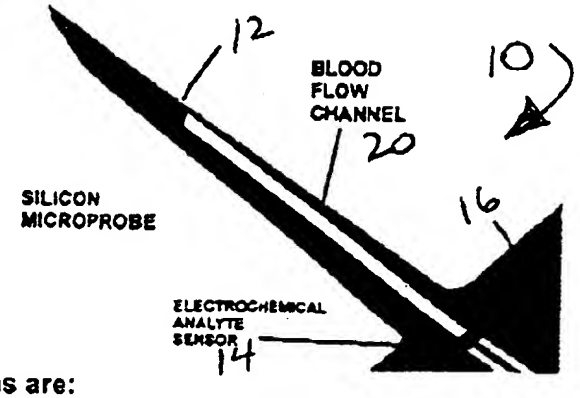


FIG. 3

Approximate dimensions are:

- Length of the microprobe = 2 to 2.5 mm
- Thickness of the microprobe = 50 to 100 μm
- Silicon device body = 1 mm square
- The microprobes will be tapered and the junction with the device body will be rounded as in the microprobes and microneedles we are currently making (see bottom devices of Fig. 4)

The device with the biosensor located *ex vivo* is a back-up design. We have noticed in our clinical trials that the microneedles and microprobes usually emerge from the skin covered with a film of blood, suggesting that analyte measurements could be made with an *ex vivo* sensor, if it is located just outside of the surface of the skin when the probe is fully inserted. An open channel could be etched into the silicon microprobe to direct blood to the biosensor. The restrictions on leakage of reagents for an *ex vivo* sensor are much less strict than for an *in vivo* sensor. Some of the key specifications for the electrochemical biosensors of the biosensor microprobes are shown in the table below.

DESIGN ISSUES

APPROACH	POTENTIAL PROBLEMS	PROPOSED SOLUTIONS
Mediated enzymatic reaction	Leakage of the mediator	Covalently bound mediator, Benign mediator, Charged membranes to reject ions
Non-mediated enzymatic reaction	Interference by ascorbate, urate, and acetaminophen Oxygen deficit	Differential current measurement (2 anodes), Improved membranes Improved diffusion control membrane
In vivo location	Biocompatibility	Improved outer membrane
Ex vivo location	Microfluidics	Improved microfluidic channels

FABRICATION ISSUES

APPROACH	POTENTIAL PROBLEMS	PROPOSED SOLUTIONS
Patterning & depositing metals	None anticipated	Use of MEMS processes
Printing with organic liquids	Spatial resolution, Drop size	High resolution ink jet printing
Forming membranes in place	Malformation	Solvent evaporation, Cross-linking, Electropolymerization
Immobilizing enzymes, mediators, and other reagents	Loss of activity, Leakage	Covalent bonding, Matrix entrapment, Chemical modification of the enzyme or mediator

Sketches showing the detailed construction of the biosensors on the silicon microprobe chips are shown below, for biosensors employing redox mediation (preferred), and a back-up design without mediators. For continuous monitoring, the biosensor microchip will be packaged with a telemetry chip to transmit analyte data to the insulin pump for closed loop control. This small button-like device (dimensions of a few millimeters) will be attached to the skin in a location such that normal movement and daily activities are not impeded, as illustrated in the following sketch.

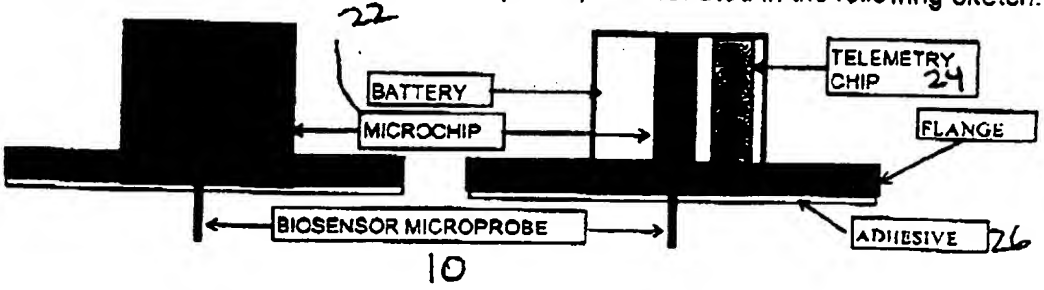
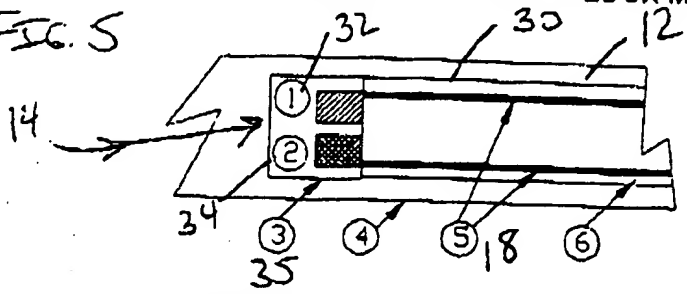


FIG. 4

ELECTROCHEMICAL CELL WITH REDOX MEDIATION

FIG. 5



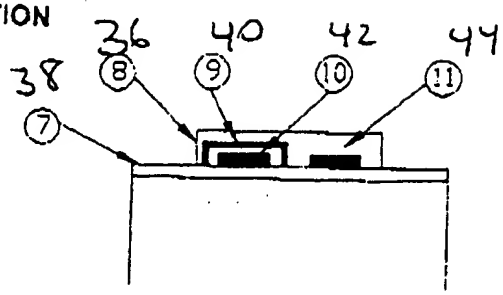
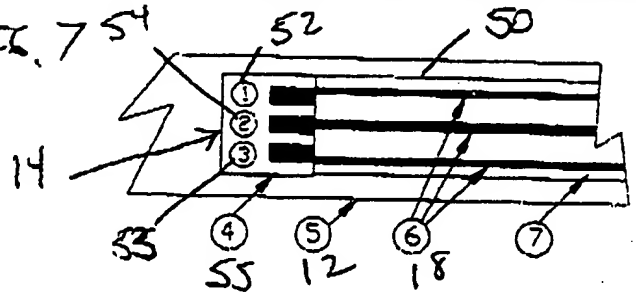


FIG. 6

1. **ANODE** Noble metal, silicon, or carbon covered with enzyme and mediator and with thin film membrane for diffusional control
2. **CATHODE** Reference electrode, such as silver - silver choloride
3. Electrolyte and outer membrane
4. Silicon microneedle
5. Electrically conducting traces
6. Protective coating
7. Oxide film
8. Outer membrane
9. Anode membrane
10. Enzyme and mediator
11. Electrolyte (Hydrogel)

ELECTROCHEMICAL CELL WITHOUT REDOX MEDIATION (BACKUP DESIGN)

FIG. 7



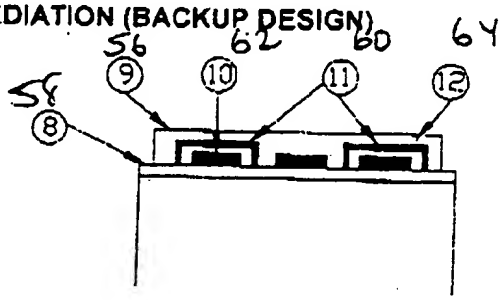


FIG. 8

1. **ANODE WITH ACTIVE GLUCOSE OXIDASE ENZYME**
   Noble metal, silicon, or carbon covered with active enzyme
2. **CATHODE** Reference electrode, such as silver - silver choloride
3. **ANODE WITHOUT ACTIVE GLUCOSE OXIDASE ENZYME**
   Noble metal, silicon, or carbon covered without active enzyme
4. Electrolyte and outer membrane
5. Silicon microneedle
6. Electrically conducting traces
7. Protective coating
8. Oxide film
9. Outer membrane
10. Active enzyme
11. Anode membranes
12. Electrolyte (Hydrogel)